

Application Serial No. 10/554,290
Amendment dated 3 March 2008
Reply to Office Action mailed 3 December 2007

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at page 17, lines 8-14 of the specification with the following amended paragraph.

Examples of flocculants that can be used include, without limitation synthetic flocculants, such as polyacrylamides, quaternary acrylate salts and natural flocculant macromolecules such as chitosan, a natural polymer derived from chitin. Particular examples of flocculants include polyacrylamide (PAM) flocculants such as Tramfloc® TRAMFLOC® (Tramfloc Inc.), the cationic flocculant SURFLOC® 34030 (Jes-Chem Ltd.), ~~polyacrylamide (PAM) flocculants such as an Aquamark® AQUAMARK® AQ 600 Series flocculant, or a SuperFloc® SUPERFLOC® C-500 Series flocculant (QEMI Inc.).~~

Please replace the paragraph at page 18, lines 13-27 of the specification with the following amended paragraph.

In the second step, any starch or related material that is present may be digested using an enzyme, such as, but not limited to an amylase. The enzyme may be used at a concentration of from about 0.05% to about 0.20% (vol/vol), from about 0.09% to about 0.15% (vol/vol), or from about 0.09% to about 0.11% (vol/vol). If an amylase is used, it is preferred that the alkaline solution be brought to an approximately neutral value of pH (i.e. ~pH 7) before adding the amylase. In an example, the solution containing the amylase is heated to a temperature of from about 50°C to about 100°C, or from about 70°C to about 90°C for about 20 to about 30 minutes to gelatinize the starch. The amylase will hydrolyse the starch and any related material. Generally, the amylase that is chosen to break down the starch material should be functional and stable within the temperature ranges indicated above. It is particularly preferred that the amylase not require a calcium co-factor to digest the starch material. Examples of such an amylase, include, without limitation, Termamyl®

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TERMAMYL® LC (Novozymes A/S), an α -amylase enzyme for starch liquefaction at low calcium levels, and Spezyme® SPEZYME® FRED (Genencor International Inc.), a starch hydrolyzing α -amylase with high heat and low pH stability.

Please replace the paragraph at page 19, lines 5-12 of the specification with the following amended paragraph.

The resulting acidified solution can then be filtered to remove any particulates and microbiological contaminants, through a filter pad that preferably has a cutoff point of about 20 mm. This filter may be coated with a pre-coat of a filter aid having a thickness of about 2 to about 5 mm, such as Celite® CELPURE® C65 (diatomaceous earth having a permeability of 0.065 Darcy; World Minerals), which has a nominal porosity of about 0.2 μ m. An equivalent weight of a filter-aid, for example, an acid-washed pharmaceutical grade filter-aid, such as Celite® CELPURE® C300 (diatomaceous earth having a permeability of 0.300 Darcy; World Minerals), may also be added as a body feed to the acidified solution prior to conducting the filtration step.

Please replace the paragraph at page 20, lines 25-29 of the description with the following amended paragraph.

To prevent gellation of the cereal β -glucan at each of the steps of the purification method of the present invention, it is preferred that the addition of salts be minimized throughout the process. For example, it is preferred that reverse osmosis (RO) purified or deionized (DI) water be used, as well as an amylase not requiring a calcium cofactor, such as Termamyl® TERMAMYL® LC (Novozymes A/S).

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Please replace the paragraph at page 24, line 14 to page 25, line 9 of the specification with the following amended paragraph.

Oat bran (The Quaker Oats Company) was slurried with alkaline reverse osmosis (RO) water at a pH of about 9.5 to a final solids concentration of 4-10%. The temperature was maintained at $45^{\circ}\text{C} \pm 5^{\circ}\text{C}$. The cereal β -glucan was extracted from the oat bran over a period of 30 minutes. After this time, the solids were removed by centrifugation with a decanter centrifuge. The centrate was cooled to room temperature, and the cationic flocculant SURFLOC[®] 34030 (Jes-Chem Ltd.) was added at a 0.2% concentration. Following an incubation period of 20 minutes, coagulated particulate material was removed by centrifugation using a disk-stack centrifuge. The pH of the centrate was adjusted to approximately neutral, heated to $>72^{\circ}\text{C}$ to gelatinize starch, and treated with the heat-stable amylase ~~Termamyl[®]~~ TERMAMYL[®] LC (Novozymes A/S). When the solution no longer produced a positive iodine test, the pH was reduced to about 4.0 to inactivate the enzyme, and the mixture was heated to 85°C for 30 minutes to denature the protein present. The solution was cooled to 4°C for one hour, and then heated to a temperature of about 72°C . An equivalent weight of ~~CELITE[®]~~ CELPURE[®] C300 (diatomaceous earth having a permeability of 0.300 Darcy; World Minerals) was added to the solution, and the mixture was then filtered using a filter-press containing 25 μm filter-papers and pre-coated to a depth of about 4 mm with ~~CELITE[®]~~ CELPURE[®] C65 (diatomaceous earth having a permeability of 0.065 Darcy; World Minerals). The filter press was preheated to a temperature of about 65°C , and the pH of the feedstream for the filter press was adjusted to 4.5 before the β -glucan solution was filtered. After the β -glucan solution was passed through the filter, the press was flushed with reverse osmosis water resulting in a clear, pale yellow coloured β -glucan solution. The β -glucan solution was cooled to 5°C and 95% ethanol at a temperature of -20°C was added to a final volume of about 15% (w/w) with stirring. A suspension of β -glucan was formed that was immediately separated from the solution by centrifugation with a disk-stack centrifuge. The isolated solid β -glucan was added to RO water at 45°C , allowed to disperse and then heated to between 60 - 70°C to produce a clear colorless solution containing about

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1% β -glucan. The separated β -glucan was colourless, had a purity of greater than 75%, a viscosity >500 cP, and an exception clarity <50 NTU, as measured using a turbidity meter.

Please replace the paragraph at page 26, lines 8 to 24 of the specification with the following amended paragraph.

At the end of the incubation period, swab samples of the skin sections were taken with both dry cotton gauze swabs and cotton gauze swabs moistened with 0.2 mL of 70% methanol/H₂O. The skin sections were removed from the **Phacocell™ PHACOCELL®** chamber and immediately frozen in liquid nitrogen. The skin sections were then cut into 15 mm slices from the horny layer to the deeper dermis. The skin sections were allowed to air dry on clean glass slides and not fixed with any fluid. The slices were then stained with BACTIDROP™ Calcofluor White for 30 seconds and then washed of excess stain with deionized water. The staining and washing steps were repeated twice. The stained sample was covered with a clean glass cover slip and examined by fluorescence with a LEIKA® fluorescent microscope having an exciter filter ranging between 400-500 nm with a peak of 440 nm, a barrier filter of 500-520 nm, and a xenon arc (burner) lamp. BACTIDROP™ Calcofluor White is a non-specific fluorochrome that binds to cellulose, and upon excitation with long wavelength ultraviolet light delineates the cell walls of cellulose-containing organisms. The deposition of the β -glucan molecules was monitored and quantified using bright fluorescence, focus inverted to white spots (3 – 5 μ m) seen upon the cell walls of the samples and in the intercellular interstices.